nature research

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.			
n/a	Confirmed				
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement			
	\square	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.			
\boxtimes		A description of all covariates tested			
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons			
		A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)			
		For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable</i> .			
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated			
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.			

Software and code

Policy information about availability of computer code									
Data collection	Nanoscope Software v9.1 (Bruker), Zen Blue 3.1 (Zeiss)								
Data analysis	Nanoscope Analysis v2.0 (Bruker), Origin Software 2017 S1 (OriginLab), GraphPad (Prism 8)								

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The Source data underlying Figs 2c,e-h; 3f,h; 4b-c; 5a and Supplementary Figs 2; 6; 7 are provided as a Source Data file. All other relevant data are available from the corresponding authors upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.							
Sample size	Typically, one hundred data points are needed for single-molecule experiments. Here we are always above this value.						
Data exclusions	No data was excluded from the analysis.						
Replication	The experiments were sucessfully replicated at least 3-5 times						
Randomization	AFM probe stochastic events, meaning random						
Blinding	No blinding was done but experiments were carried out and analyzed by two different operators						

Reporting for specific materials, systems and methods

Methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	n/a	Involved in the study
\ge	Antibodies	\boxtimes	ChIP-seq
	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
\boxtimes	Animals and other organisms		
\boxtimes	Human research participants		
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		

Eukaryotic cell lines

Policy information about <u>cell lines</u>						
Cell line source(s)	A-549 cell line was purchased from ATCC (ATCC® CCL-185)					
Authentication	The cell line was obtained from ATCC and authenticated prior to purchase.					
Mycoplasma contamination	We did not see any indicators of mycoplasma contamination.					
Commonly misidentified lines (See <u>ICLAC</u> register)	The cell line used is not listed as commonly misidentified in the ICLAC register.					